Biogeochemistry of Hypersaline Microbial Mats Illustrates the Dynamics of Modern Microbial Ecosystems and the Early Evolution of the Biosphere

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Photosynthetic microbial mats are remarkably complete self-sustaining ecosystems at the millimeter scale, yet they have substantially affected environmental processes on a planetary scale. These mats may be direct descendents of the most ancient biological communities in which even oxygenic photosynthesis might have developed. Photosynthetic mats are excellent natural laboratories to help us to learn how microbial populations associate to control dynamic biogeochemical gradients.

Light sustains both oxygenic and anoxygenic photosynthesis; in turn, photosynthesis provides energy, organic substrates, and oxygen to the community (Fig. 1). Although photosynthetic bacteria might dominate the biomass and productivity of the mat, many aspects of the emergent properties of this ecosystem ultimately reflect the activities of the associated nonphotosynthetic microbes, including the anaerobic populations. These nonphotosynthetic processes constitute the ultimate biological filter on chemical biomarkers (*e.g.*, porphyrins, hopanes, isoprenoids, and other biogenic hydrocarbons), and also on isotopic and geologic biosignatures that subsequently enter the fossil record. Also, the transformation of photosynthetic productivity by the microbial community can contribute diagnostic "biosignature" gases that might represent examples of search targets for remote detection of astronomical life (*e.g.*, Des Marais *et al.*, 2002a). To understand the overall structure and function of mat communities, it is thus critical to determine the nature and extent of interaction between photosynthetic and nonphotosynthetic, including anaerobic, microbiota.

Both the diversity of biota and the functional complexity within the mats, coupled with the highly proximal and ordered spatial arrangement of microorganisms, offer the potential for a staggering number of interactions. The products of each group can affect the responses of other groups in both positive and negative ways. For example, cyanobacteria generate organic matter (a potential growth and energy substrate for other organisms) but also oxygen (a toxin for many anaerobic processes). Anaerobic activity recycles nutrients to the phototrophic community, but it also generates potentially toxic sulfide (Van Gemerden, 1993). Accordingly, microorganisms have developed strategies to cope with the daily oscillation between extremes of eutrophy and toxicity.

A study of subtidal cyanobacterial mats growing in the hypersaline seawater evaporation ponds of the salt producer Exportadora de Sal, S.A. (ESSA) is ongoing at Guerrero Negro, in Baja California, Mexico. This study is furnishing examples of the steep environmental gradients experienced by mat microorganisms, and providing preliminary evidence for intimate interactions between these populations. These observations indicate that future studies of genome-genome interactions will contribute substantially to our understanding of the origins, environmental impacts, and biosignatures of photosynthetic microbial mats.

The Microenvironment Within Photosynthetic Microbial Mats

To understand the function of a microbial mat community, the physical and chemical microenvironment in which the microbes live must be known well and in detail. At Guerrero Negro, the environment within the mat differs substantially from that in the overlying water column. The
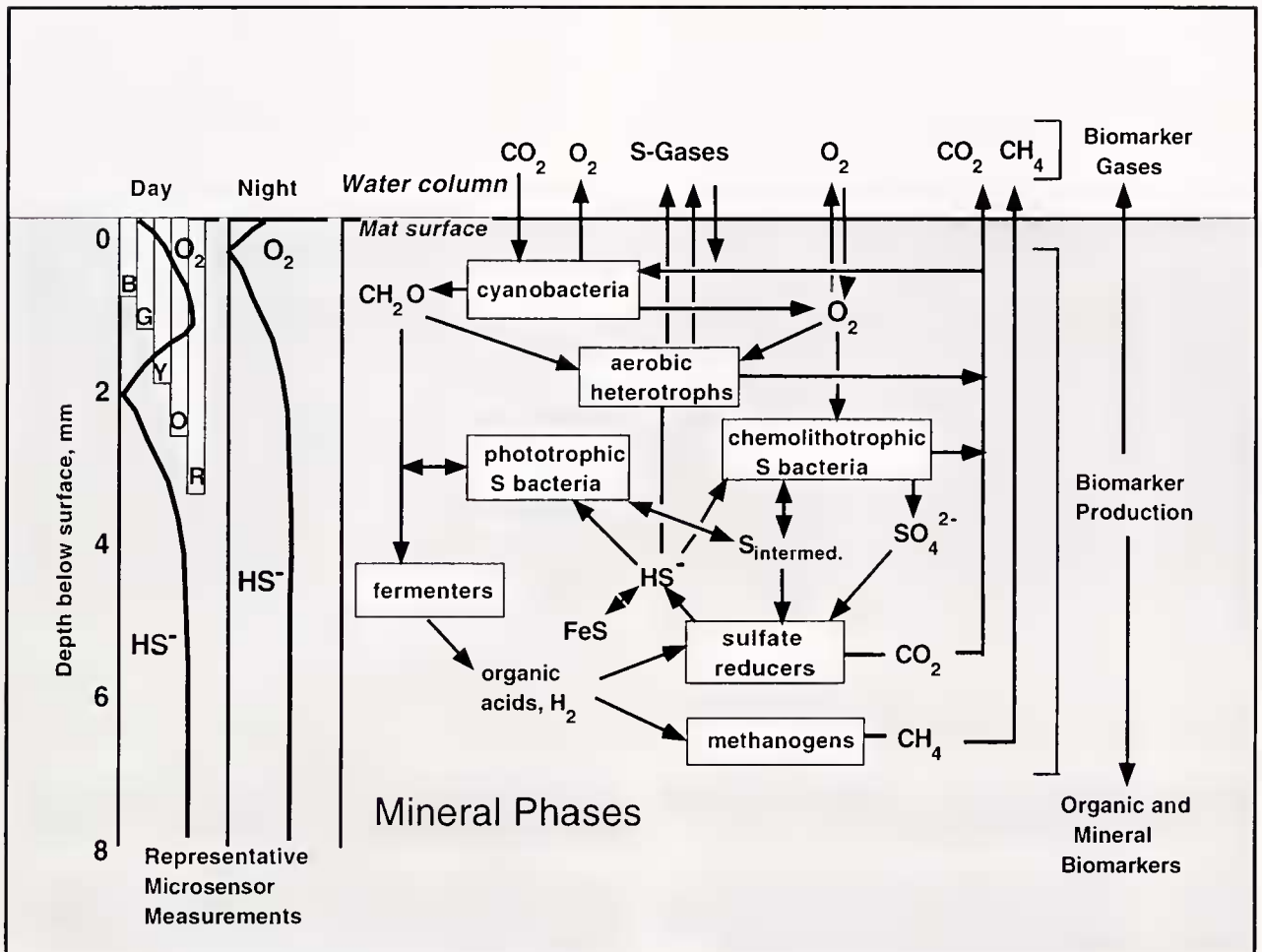
Figure 1. Schematic of a cyanobacterial microbial mat with associated depth-related light and chemical gradients. Flow diagram at center is modeled after Fenchel and Finlay (1995). Boxes denote functional groups of microorganisms, and arrows denote flows of chemical species into or out of microorganisms. $S_{intermed}$ indicates sulfur in intermediate oxidation states. Schematic at left depicts vertical gradients of O_2 and sulfide during the day and at night. Oxygen concentrations are shown decreasing to zero at a depth of 2 mm during the day, and just below the mat surface at night. The vertical bars at upper left represent the relative depths of penetration of blue (B), green (G), yellow (Y), orange (O), and red (R) light. Such chemical gradients and light penetration profiles of both filamentous and unicellular mats are qualitatively similar, although the depth scale (mm) of such profiles tends to be greater for unicellular mats.

community just beneath the mat surface typically experiences steep vertical gradients of light intensity and redox conditions that change markedly during the diel cycle.

The intensity and spectral composition of the light that penetrates the mat is changed by absorption and scattering. Motile photosynthetic organisms optimize their position with respect to the resultant light gradient; some biota harvest light in the infrared spectral range (Jørgensen et al., 1987; Jørgensen and Des Marais, 1988). When oxygenic photosynthesis ceases at night, the upper layers of the mat become highly reduced and sulfidic (Jørgensen et al., 1979; Jørgensen, 1994). Counteracting gradients of oxygen and sulfide shape the chemical environment and provide daily-contrasting microenvironments that are separated on a scale of a few millimeters (Fig. 1; Revsbech et al., 1983). Radi-

ation hazards (UV, etc., Garcia-Pichel, 2000) as well as oxygen and sulfide toxicity elicit motility and other physiological responses. This combination of benefits and hazards of light, oxygen, and sulfide promotes the allocation of the various essential mat processes to the periods of light and dark periods (Fründ and Cohen, 1992; Bebout et al., 1994) and to various depths in the mat.

Light microenvironment

The light flux penetrating the mat can be measured both as downward irradiance (the total down-welling light that passes through a horizontal plane) and as scalar irradiance (the sum of all light that converges upon a given point within the mat). Due to the high density of photosynthetic

organisms, bacterial mucilage, and mineral particles in mats, light absorption is dominated by the light-harvesting pigments of the phototrophic bacteria, and light is strongly scattered. Because absorption and scattering of light are quite substantial within the mat, scalar irradiance can differ substantially from downward irradiance (Jørgensen and Des Marais, 1988). Because scalar irradiance measures the total light actually available at a given location, it constitutes the most meaningful description of the environment of a microorganism.

Measurements of scalar irradiance were obtained both for a microbial mat that was dominated by a filamentous cyanobacterium, *Microcoleus chthonoplastes,* and for a mat that grew at higher salinity and was dominated by unicellular cyanobacteria (Jørgensen and Des Marais, 1988). A strong decline in intensity and a marked change in spectral composition of the light are both typically observed with depth in the dark olive mat, dominated by *Microcoleus* cyanobacteria. Minima in the spectra correspond to the absorption maxima of the photosynthetic pigments of cyanobacteria. Chlorophyll *a* (Chl *a*) absorbs at wavelengths of about 430 and 670 nm, phycocyanin at about 620 nm, and various carotenoids in the range of 450 to 500 nm. In contrast, the mat that was dominated by unicellular cyanobacteria had a lower density of cells, a more gelatinous texture, and a light orange-tan color. Light penetrated more deeply into the unicellular cyanobacterial mat, although blue light was strongly attenuated. Carotenoids achieved most of the light absorption in this mat. In both the *Microcoleus* and unicellular mats, longer-wavelength light, particularly longer than 900 nm, penetrated farthest into the mat (Fig. 1).

Such studies illustrate how the mat matrix affects the penetration of light and the physiology of the biota. For example, mat cyanobacteria that use light that has been filtered by overlying diatoms exhibit greatest photosynthetic activity at wavelengths between 550 and 650 nm (Jørgensen *et al.,* 1987), a region that lies between the absorption maxima of Chl *a.* In contrast, planktonic cyanobacteria exposed to a broader spectrum of light in their natural environment show significant activity at wavelengths corresponding to the absorption maxima of Chl *a* (Jørgensen and Des Marais, 1988).

Chemical gradients

The high rates of oxygenic photosynthesis that occur in the narrow photic zone of the mat create steep and variable gradients (Revsbech *et al.,* 1983) in pH and in concentrations of dissolved inorganic carbon (DIC) and O_2 (DO). The oxic zone reflects a dynamic balance between photosynthetic O_2 production and O_2 consumption by a host of sulfide-oxidizing and heterotrophic bacteria.

Using microelectrodes, the depth distribution of [DO], sulfide concentrations, and pH was determined in a mat dominated by *M. chthonoplastes* (Jørgensen and Des Marais,

1986). These depth profiles are typical for these mats (*e.g.,* Canfield and Des Marais, 1993). Extremely high rates of oxygenic photosynthesis create DO levels that are nearly five times the value of air-saturated brine, yet this O_2 has a residence time of only 2 min. Oxygen production can become negligible at a depth of only 0.5 mm, due to light limitation (Fig. 2a). However, O_2 diffuses farther down to a point at which it overlaps with sulfide diffusing up from below. This interval is typically inhabited by abundant green nonsulfur phototrophic bacteria (*e.g., Chloroflexus*) and by *Beggiatoa* (Fig. 3b). As sunset approaches, the oxic zone collapses quickly, and the oxic-anoxic boundary approaches the mat surface (Canfield and Des Marais, 1993). Accordingly, conditions alternate between O_2 supersaturation and millimolar concentrations of sulfide. Remarkably, diverse microbiota have apparently become well adapted to these conditions.

The relative abundances of photoautotrophic bacteria (*e.g.,* purple sulfur bacteria and green nonsulfur bacteria) and chemolithotrophic sulfide-oxidizing bacteria can be affected by the amount of light that reaches the chemocline (Jørgensen and Des Marais, 1986). Light levels as low as 1% of the incident near-infrared radiation (800 to 900 nm) are sufficient for *Chromatium,* a phototrophic purple sulfur bacterium, to dominate the chemocline (Fig. 2b). Thus the balance between the penetration of O_2 and the penetration of light into the sulfide-rich zone determines the balance in the populations of sulfur bacteria.

Biogeochemical Cycling of Key Elements and Their Compounds

The waters that host well-developed microbial mats are typically depleted in the basic nutrient elements (Javor, 1983), yet microbial mats are highly productive aquatic ecosystems. This remarkable productivity reflects the efficient recycling of key nutrients within the mat ecosystem. The cycling of carbon, oxygen, sulfur, and nutrients has been studied in mats dominated by *M. chthonoplastes* (*e.g.,* Canfield and Des Marais, 1993; Bebout *et al.,* 1994).

The potential for substantial coupling among populations arises through cyanobacterial production of hydrogen and small organic acids (Fig. 1; Stal *et al.,* 1989; Van Der Oost *et al.,* 1989; Stal, 1991). Such substances can serve as substrates for energy and growth for a broad array of microorganisms. Bacterial production of low-molecular-weight nitrogen and sulfur compounds is also important. These compounds lie at the center of energy and electron flow in anaerobic ecosystems and thus are potential basis for microbial interactions. For example, interspecies transfer of hydrogen facilitates many well-studied anaerobic consortia (*e.g.,* Ferry and Wolfe, 1976; McInerney *et al.,* 1979). Hydrogen can represent not only an agent of electron transfer but also an important thermodynamic control with the potential for significantly altering the metabolic function of
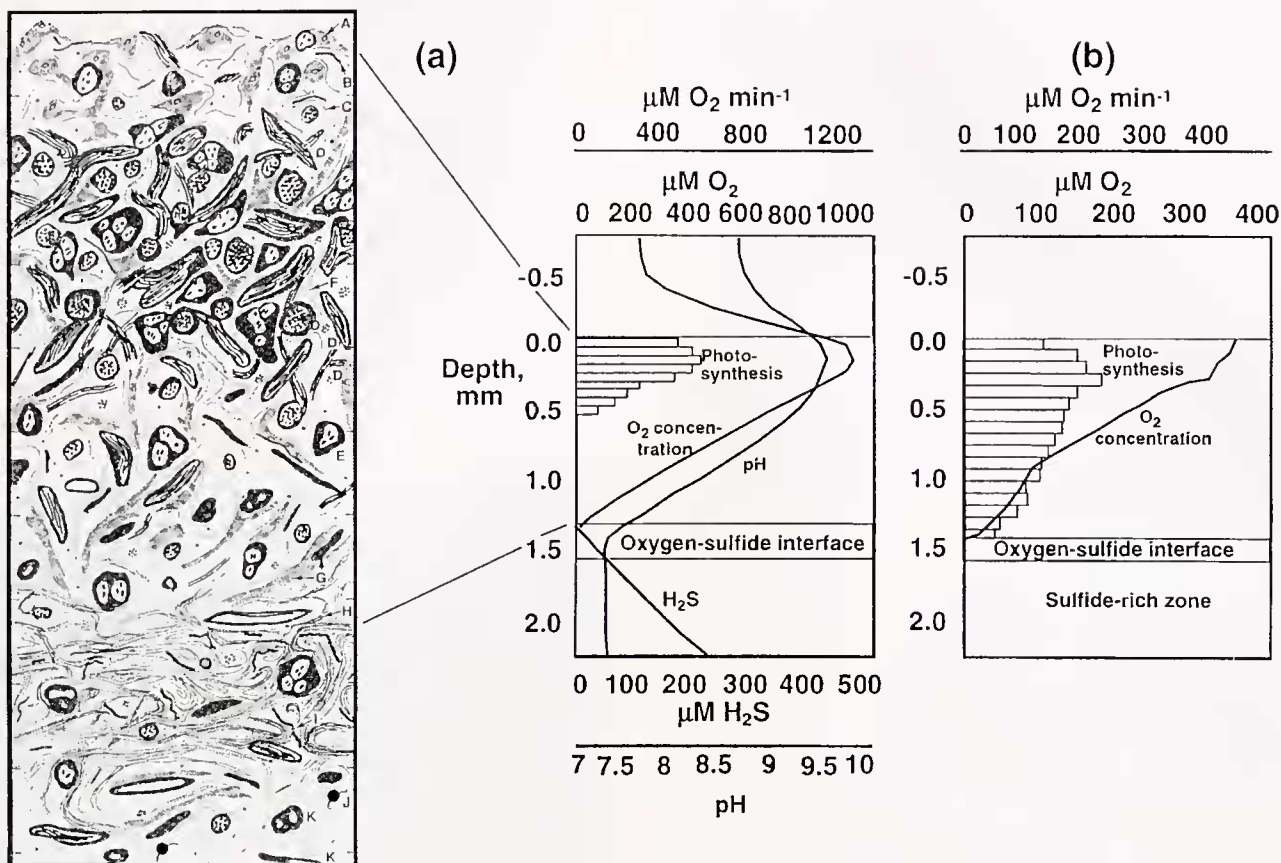
Figure 2. Depth gradients in O_2, oxygenic photosynthesis, sulfide, pH, and microbiota. (a) At left is a schematic vertical section of the topmost 2 mm of subtidal mat dominated by *Microcoleus chthonoplastes* cyanobacteria. Letters along the right margin indicate the following: A: diatoms; B: *Spirulina* spp. cyanobacteria; C: *Oscillatoria* spp. cyanobacteria; D: *Microcoleus chthonoplastes* cyanobacteria; E: nonphotosynthetic bacteria; F: unicellular cyanobacteria; G: fragments of bacterial mucilage; H: green nonsulfur bacteria; I: *Beggiatoa* spp.; J: metazoans (*e.g.*, nematodes); K: abandoned cyanobacterial sheaths. Also shown are depth profiles for key chemical constituents in the Pond 5 mat, as follows: photosynthetic O_2 production rates (horizontal bars), and concentrations of O_2 and sulfide, and pH (data from Jørgensen and Des Marais, 1986).

each partner. Virtually every member of the anaerobic community is subject to such effects (Schink, 1988; Zinder, 1993); therefore, the participation of cyanobacteria in the cycling of hydrogen and organic acids could substantially affect biogeochemical function and community composition (Hoehler *et al.*, 2001). Similarly, anaerobes consume these thermodynamically sensitive end products and thus can provide an important feedback on fermentation and nitrogen fixation by cyanobacteria at the levels of both enzyme and gene regulation.

Carbon, oxygen, and sulfur budgets

Several general observations can be made about the cycling of carbon, oxygen, and sulfur (Canfield and Des Marais, 1993; Des Marais *et al.*, 2002b). During the day, most of the O_2 produced is recycled within the mat by O_2 respiration and some sulfide oxidation. At night, O_2 is consumed principally by sulfide oxidation near the mat-water interface. Microbial sulfate reduction is the principal source of DIC at night. Although abundant *Chloroflexus*-type (anoxic phototroph) filaments are visible microscopically at the O_2-sulfide interface, anoxygenic photosynthesis accounts for less than 10% of the total carbon fixation rate.

A careful comparison of the relative O_2 and DIC fluxes across the mat-water interface reveals that, during the day, more DIC diffuses into the mat than O_2 diffuses out (Canfield and Des Marais, 1993; Des Marais *et al.*, 2002b). At night, more DIC diffuses out of the mat than O_2 diffuses in. However, both the net O_2 and the net DIC fluxes are balanced over the full 24-h cycle. This budget indicates that, during the day, carbon having an oxidation state greater than zero is incorporated into the mat, and carbon having a similarly high oxidation state is liberated at night. The chemical nature of this carbon is unknown.

Although all of the key processes are strongly influenced by temperature, their rates scale with temperature by

roughly the same amount (Canfield and Des Marais, 1993; Des Marais *et al.*, 2003). Over a 24-h period, the overall impact of these very high metabolic rates is that the net accumulation of carbon is low. Apparently this mat is a closely coupled system in which high rates of photosynthetic carbon fixation fuel high rates of carbon oxidation. This efficient oxidation of organic components regenerates nutrients that, in turn, maintain high rates of primary production.

Gas production

The high productivity associated with photosynthetic microbial mats, coupled with their proximity to the atmosphere and prominent role in ancient coastal environments, indicates that such mats probably influenced the early atmosphere substantially. Cyanobacteria and diatoms provide large quantities of photosynthate to anaerobes in the mat. In the *Microcoleus* mats at night, O_2 is consumed by sulfide oxidation at the mat surface and lowermost water column (Canfield and Des Marais, 1993), thus the entire mat becomes anoxic (Fig. 1). Accordingly, mat cyanobacteria must ferment to obtain energy at night, and they probably produce an array of reduced low-molecular-weight compounds. At Guerrero Negro, Hoehler *et al.* (2001) observed that subtidal *Microcoleus* mats generated CO, CH_4, and significant quantities of H_2. Rates of emission of CO correlated with rates of photosynthesis, implicating cyanobacteria, diatoms, or both as sources. Emission rates of H_2 were greatest at night, consistent with fermentation under anoxic conditions. Methane emission rates were unchanged during the diel cycle, indicating a source beneath the zone in the mat that becomes oxygenated during the day. Abundant organic photosynthates apparently interact also with sulfides in mat pore waters to produce dimethyl sulfide and other organosulfur gases, some of which escape the mat (Visscher *et al.*, 2003).

These fluxes of reduced gases are significant for at least three reasons. First, microorganisms that inhabit cyanobacterial mats benefit from abundant products of photosynthesis. Therefore, the advent of oxygenic photosynthesis billions of years ago perhaps triggered a profound evolutionary transformation and diversification within the anaerobic microbial world. Second, the proximity of photosynthetic mats to the atmosphere allows a substantial fraction of reduced gases to escape biological recycling and to enter and profoundly alter atmospheric composition (Hoehler *et al.*, 2001). Early in Earth history, atmospheric reduced biogenic gases such as methane and organosulfides might have been important both as greenhouse gases and as substrates for energy and growth of other biota, including those that were geographically distant from the sources of these gases. Third, if analogous microbial ecosystems indeed exist on habitable planets orbiting other stars, they should influence the compositions of their atmospheres. The closest of these planets might soon be observable by astronomers (*e.g.*, Des Marais *et al.*, 2002a).

Specific Microbe-Microbe Interactions

The mat ecosystem depends upon intimate interactions between key groups of bacteria. Oxygenic photosynthesis by cyanobacteria and diatoms maintains a "food chain"— that is, a flow of both photosynthetic products and their derivatives that nourishes a vast consortium of mat microorganisms (*e.g.*, Fig. 3). The specific details of this flow of reduced species are so important that they might account for several key unanticipated observations; two such examples are described below.
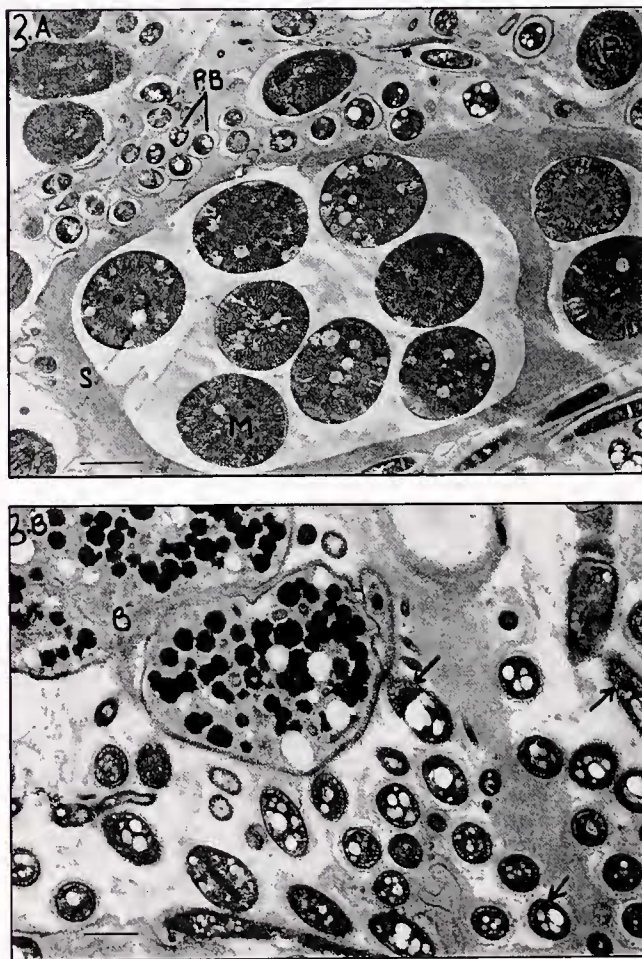

Figure 3.   Transmission electron micrographs of biota in the subtidal *Microcoleus* mat (Elisa D'Antoni D'Amelio, Ames Research Center, unpubl.). (3A) Community at about 0.2 mm depth, showing several *Microcoleus* (M) trichomes situtated within a common exopolymer sheath (S). Nearby are *Phormidium* cyanobacteria (P) and anoxygenic phototropic bacteria (PB), possibly green nonsulfur bacteria. Scale bar at lower left equals 1 μm. (3B) Community at about 1.4 mm depth, just below the O_2 sulfide chemocline. Large *Beggiatoa* filaments (B) are accompanied by photosynthetic green nonsulfur bacteria exhibiting their characteristic intracellular photosynthetic bodies, chlorosomes (arrows). Scale bar at lower left equals 1 μm.

Association between cyanobacteria and anoxygenic phototrophic bacteria

An unidentified filamentous phototrophic bacterium has been described which actually lives inside the sheaths of viable *Microcoleus* cyanobacteria (D'Amelio *et al.*, 1987). Its occurrence inside cyanobacterial sheaths is interesting because O_2 inhibits anoxygenic photosynthesis. D'Amelio *et al.* (1987) proposed that, because light levels and DO vary during the day, this bacterium alternates between photoheterotrophic growth (at high light levels; using organic matter excreted by the cyanobacteria) and sulfide co-metabolism with cyanobacteria (at relatively low light levels, where O_2 production is minimal). This anoxygenic phototroph might even assist cyanobacteria by consuming sulfide after sunrise and thus relieving sulfide inhibition of oxygenic photosynthesis.

Aerobic sulfate reduction

Sulfate-reducing bacteria are quantitatively important consumers of dissolved organic matter. Furthermore, the sulfide they produce sustains a wide variety of phototrophic and chemotrophic bacteria. The highest rates of sulfate reduction occur in the shallowest part of the subtidal *Microcoleus* mat, close to the photosynthetic source of fresh organic matter (Canfield and Des Marais, 1993). Although O_2 is typically an effective inhibitor of bacterial sulfate reduction, the highest reduction rates actually occur within the mat's aerobic zone during the daytime (Canfield and Des Marais, 1991). A thorough search was made for anaerobic microenvironments within the aerobic zone that might serve as refugia for the sulfate-reducing bacteria, yet none were found. The specific factors that attenuate this O_2 inhibition of sulfate reduction are not known. However, fermentation products are probably abundant in the vicinity of the cyanobacteria, and their roles as chemical reductants might offset the toxic effects of oxidants such as O_2.

Future Research

The studies described above have been performed principally on subtidal marine hypersaline cyanobacterial mats. Although intertidal and supratidal cyanobacterial mats also have received some attention, the level of effort summarized in this review must continue to be applied to other mat types. Examples of such mats include those dominated by unicellular cyanobacteria, eukaryotic algae, and nonphotosynthetic bacteria such as sulfide-oxidizing bacteria in deepsea communities (*e.g.*, Ward *et al.*, 1992; Stal and Caumette, 1994). Mats growing in low-sulfate environments such as lakes, streams, and thermal springs also merit more attention.

Studies of biogeochemical cycling in mats should be broadened to include additional populations of mat microorganisms (*e.g.*, heterotrophs, methanogens, and novel bacteria) that probably contribute substantially to the community. We must better understand how key nutrients such as nitrogen and phosphorus are regenerated and retained by the various types of mats. Mats that coexist with active mineral precipitation (*e.g.*, with calcium carbonate: Fouke *et al.*, 1999; Reid *et al.*, 2000) merit study to help us understand the roles of the microbes in the precipitation of minerals and the impact of mineral formation upon mat biogeochemistry.

Perhaps most promising is the use of gene sequences and gene expression studies to understand the ecology of microbial mat communities. Methods for the identification and interpretation of 16s RNA and other macromolecules are improving rapidly and hold great promise. These phylogenic investigations should be combined with studies of biogeochemistry and gene expression to elucidate the key linkages between microbial populations, processes, and the emergent products and environmental impacts of microbial mats.

Acknowledgments

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Discussion

QUESTION: As you mentioned, Dave, cylindrical sheaths made of exopolymeric material have within them two populations of organisms that you have proposed might maintain a relationship responsive to diurnal phenomena. Do you think that the mat structure is very heterogeneous, containing clusters of organisms that would, based upon our current understanding, not be expected to co-exist? For example, do some associations between populations protect organisms from harmful products?

DES MARAIS: Yes, I included these examples of associations between populations of organisms to illustrate this point. I think also that this is one of the reasons why Mitch Sogin had me talk early in this meeting; that is, to show that there is, potentially, a long menu of important ecological phenomena that could be addressed by genomic studies. Again, in the example I showed, sulfide is removed in the morning by the anoxygenic phototrophic bacteria, which benefits the cyanobacteria. This example also includes the cross feeding by the cyanobacteria that benefits the anoxygenic phototrophs. Evidence for cross feeding has actually been documented in Yellowstone Park by David Ward. Using isotopic labeling, he observed that photosynthate does flow from cyanobacteria to green non-sulfur bacteria. I think we have demonstrated that sulfide inhibits at least some types of oxygenic photosynthesis in these mats. Also, anoxygenic phototrophs can reduce sulfide levels in natural environments. Another example is that the surface-dwelling microbial populations screen the deeper ones from UV and shorter wavelengths that would be injurious to their photosynthetic apparatus. Dick Castenholz, Ferran Garcia-Pichel and others have documented this extensively.

QUESTION: I am wondering whether other organisms are sequestered in a way that enhances certain processes. You presented

measurements of very high rates of sulfate reduction. Are the high rates possible because sulfate-reducing bacteria are protected by other organisms that you are just not observing directly?

DES MARAIS: I will defer to Dave Stahl to discuss current studies of bacterial sulfate reduction that occurs in the presence of molecular oxygen. But, some ten years ago, Don Canfield and I probed extensively, with electrodes, for anoxic micro-environments within the oxygenated photic zone of cyanobacterial mats. In these mat porewaters, oxygen diffuses some 50 to 100 microns in just a few seconds, and so we spaced our electrode sampling profiles about one mm apart. But we just couldn't find anoxic microenvironments. However, even though oxygen is a strong oxidant, the rate at which it oxidizes other substances is not as fast as the rates of some other oxidants, such as radicals, etc. Perhaps the deleterious oxidation reactions involving oxygen are mitigated by faster reactions carried out by reductants such as hydrogen. Relatively fast-acting reducing compounds in the mats might confer the advantage that the sulfate-reducing bacteria need to maintain these very high rates in the presence of oxygen. So, the relative rates of reactions, as well as mechanisms of physical protection, might play key roles in these mat communities.

QUESTION: What about migration? There is another dynamic with biota moving in response to chemical gradients.

DES MARAIS: What I find interesting is that some populations migrate, whereas others don't even under circumstances where migration would seem to benefit both of these populations. For example, microorganisms can migrate vertically within cyanobacterial mats in response to changes in the depth of oxygen and light penetration, which can vary diurnally by a few mm. *Beggiatoa* are nonphotosynthetic bacteria that oxidize sulfide with oxygen, and so it is advantageous for them to occupy zones where both sulfide and oxygen coexist. But, there are different diameters of *Beggiatoa*, and I recollect that the ones having smaller diameters tend to be much more mobile than the big ones. Also, some photosynthetic populations, particularly purple sulfur bacteria, have been observed to migrate. How does the community select between populations that migrate as conditions change, *versus* the more stationary populations?

There are examples of two environmental cues for migration that appear be in conflict with each other. What causes *Beggiatoa* to migrate downward after sunrise? Is it the light or is it the oxygen and sulfide chemical gradients? It can't be just an avoidance response to high oxygen concentrations. When *Beggiatoa* is still at the surface in the morning, bathed in sunlight, oxygen production is starting beneath it. At some point, *Beggiatoa* must dive down through an oxygen rich zone to get down to the darker sulfide-oxygen interface that is most suitable during the daytime. So the question "What are all of the cues that drive these organisms to migrate?" is both an important question and one that is still in search of definitive answers.